

University of Groningen

**Influence of sheep manure addition on biogas potential and methanogenic communities during cow dung digestion under mesophilic conditions**

Achinas, Spyridon; Li, Yu; Achinas, Vasileios ; Euverink, Gerrit Jan Willem

*Published in:*  
Sustainable Environment Research

*DOI:*  
[10.1016/j.serj.2018.03.003](https://doi.org/10.1016/j.serj.2018.03.003)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2018

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Achinas, S., Li, Y., Achinas, V., & Euverink, G. J. W. (2018). Influence of sheep manure addition on biogas potential and methanogenic communities during cow dung digestion under mesophilic conditions. *Sustainable Environment Research*, 28(5), 240-246. <https://doi.org/10.1016/j.serj.2018.03.003>

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



## Original Research Article

## Influence of sheep manure addition on biogas potential and methanogenic communities during cow dung digestion under mesophilic conditions

Spyridon Achinas<sup>a,\*</sup>, Yu Li<sup>a</sup>, Vasileios Achinas<sup>b</sup>, Gerrit Jan Willem Euverink<sup>a</sup><sup>a</sup> Faculty of Science and Engineering, University of Groningen, Groningen 9747 AG, The Netherlands<sup>b</sup> Union of Agricultural Co-operatives of Monofatsi, Heraklion 70016, Greece

## ARTICLE INFO

## Article history:

Received 31 October 2017

Received in revised form

25 January 2018

Accepted 15 March 2018

Available online 22 March 2018

## Keywords:

Anaerobic co-digestion

Animal slurries

Biogas potential

Methanogens

## ABSTRACT

The efficient treatment of animal slurries can support the bioenergy management and environmental protection; however, the low biogas yield and quality are the major constraints. The object of this paper is to investigate how the co-digestion of sheep manure and cow dung by not using inoculum influences the performance of the process and determine the methanogenic communities at the end of the experiment. Biochemical Methane Potential essays were conducted in mesophilic conditions in order to determine the biogas-methane potential. Enhanced biogas production was achieved from the mono-digestion of cow dung with 104.3 NmL biogas g<sup>-1</sup> VS and the co-digestion of cow dung and sterilized sheep manure with a lower biogas yield of 89.0 NmL biogas g<sup>-1</sup> VS.

© 2018 Chinese Institute of Environmental Engineering, Taiwan. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Dairy industry is now a significant global industry and it contributes to nearly 7% of total agricultural production value [1]. It is also one of the most important industries in the Netherlands. With the expansion of the big farms, a lot of herds now lead to large amount of livestock manure which causes serious environmental problems such as greenhouse gas (GHG) emissions, surface water contamination, and animal related pathogens [2,3]. Among livestock manure, the majority is produced by cattle. As ruminants (mainly dairy and beef cattle) contribute the largest proportion (61%) to livestock-related GHG emissions [4,5], there is an increased pressure to reduce their carbon footprint.

Anaerobic digestion (AD) produces biogas for heat and power as well as solid residue - so-called digestate-which can also be used as organic fertilizer in agricultural activities [6]. The basic steps of the organic mass conversion to biogas are illustrated in Fig. 1. The mono-digestion of cattle manure is proved to be reasonable because it contains bacteria needed in the fermentation phase as well as degradable materials such as carbohydrate and lipids. But on

the other hand, the fermentation of cattle manure alone often results in low biogas production and sometimes reaches only quarter of the theoretical biogas yield. Moreover, compared with other farm animals, the biogas yield of cattle is lower because of its lignin complexes from fodder that are very resistant to AD [7]. It is critical to find another proper substrate to co-digestion with cattle manure in order to balance the nutrition and dilute the limitations in the AD process.

This paper chooses sheep manure (SM) for co-digestion with cow dung (CD). Cestonaro et al. [8] previously used the co-digestion of sheep bedding with cow manure without inoculum at room temperature and found that when adding 50% or more cow manure, it would increase the biogas production and improves the digestate quality. Alvarez and Liden [9] used the co-digestion of llama, cow manure and SM for improving methane production and found that co-digestion was better than the mono-digestion among those kinds of animal manure. However, previous studies of cow manure co-digestion with SM do not take into account the AD without inoculum. So there is a need to see the performance of co-digestion of sheep and cow manure without inoculum in order to investigate the interactions of the microorganisms present in the animal slurries. The object of this paper is to investigate how the co-digestion of SM and CD by not using inoculum influences the performance of the process and determine the methanogenic communities at the end of the experiment.

\* Corresponding author.

E-mail address: [s.achinas@rug.nl](mailto:s.achinas@rug.nl) (S. Achinas).

Peer review under responsibility of Chinese Institute of Environmental Engineering.

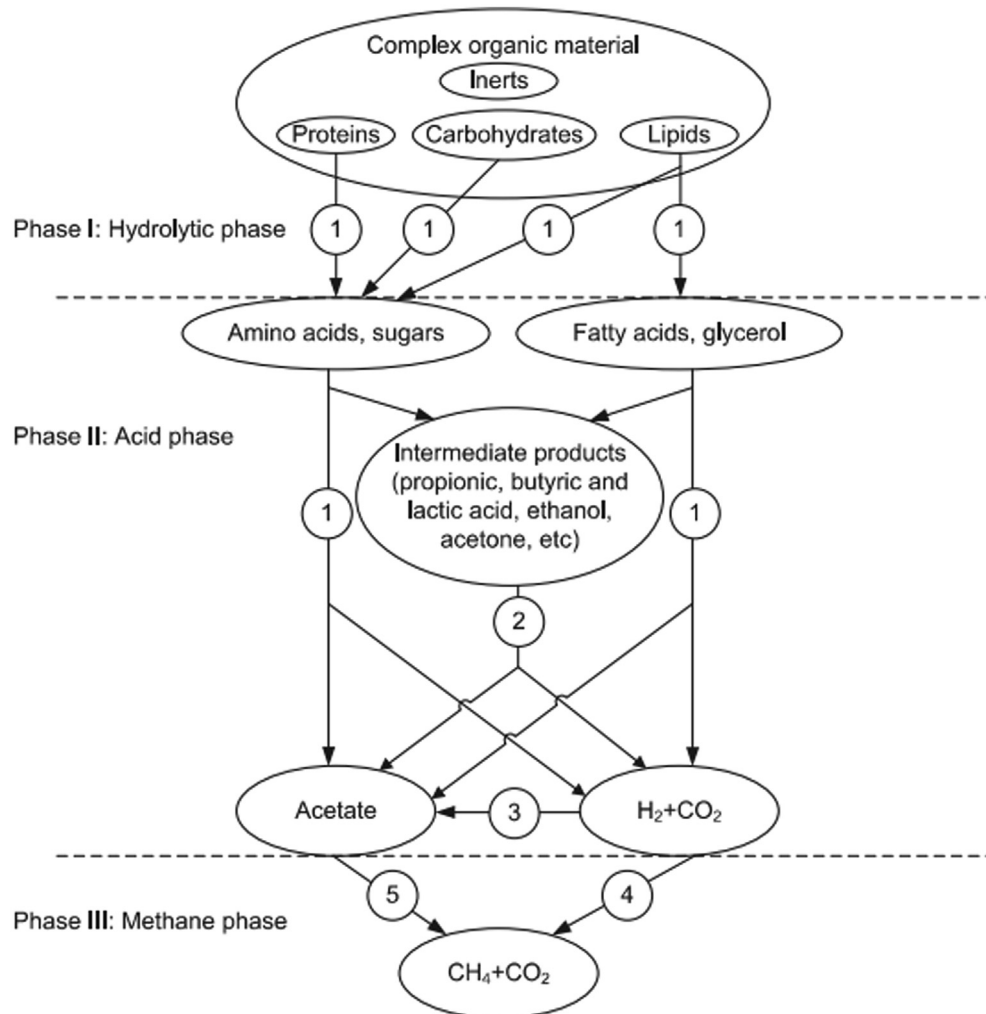


Fig. 1. Steps related with anaerobic digestion of organic materials.

## 2. Materials and methods

### 2.1. Origin of inoculum and substrates

Animal slurries were used as substrates in the experiments. Fresh CD and SM were collected from a farm in Groningen (Netherlands). Two different materials were selected for experimental essays as they are considering important source for agricultural bioenergy production. Two additional samples of cow manure and SM were undergone autoclaving (10 min) in order to eliminate the microorganisms. Pecorini et al. [10] report that short-term autoclaving does not influence the hydrolysis of cellulosic fraction of non-biodegradable substances. Their characteristics in terms of volatile solids (VS), total solids (TS) and chemical oxygen demand (COD) are given in Table 1. All the substrates were

undergone agitating for 5 min before final feed in order to increase the active surface of the particles. The substrates were stored at 4 °C prior to use.

### 2.2. Experimental essays

The experiment carried out in batch mode using the water displacement method for measuring the biogas produced. The biogas potential was based on the total volume of biogas produced during the degradation period and is defined as NmL biogas  $g^{-1}$  VS added. Fig. 2 represents the set-up employed for the experimental procedure. In our set-up, 500 mL serum bottles were used for the essays (Fig. 2), flushed with  $N_2$  for 2 min in order to maintain anaerobic conditions, placed in an incubator at a constant mesophilic temperature ( $36 \pm 1$  °C) and shaken at 150 rpm during the experimental period of the assay. Two tests studied with CD and SM digestion, the other three tests studied mixtures of CD:SM, CD:SSM (sterilized sheep manure) and SCD:SM with ratio 1:1 based on VS concentrations. Stocks samples (substrates solutions) were prepared and the serum bottles were filled starting with the substrates, followed by the addition of distilled water in order to achieve a working volume of 350 mL. No inoculum or additional external nutrients/trace elements was added to the serum bottles. The experimental conditions and the content of the reactors are given in Table 2.

Table 1  
Characteristics of the substrates.

Feedstocks*	TS (g $kg^{-1}$ )	VS (g $kg^{-1}$ )	COD (g $kg^{-1}$ )	TS/VS	COD/VS
Cow dung (CD)	121.3	107.2	134.7	1.1	1.3
Sheep manure (SM)	252.8	213.8	349.2	1.1	1.5
Sterilized cow dung (SCD)	135.4	121.7	186.6	1.2	1.6
Sterilized sheep manure (SSM)	237.4	199.0	250.2	1.2	1.3

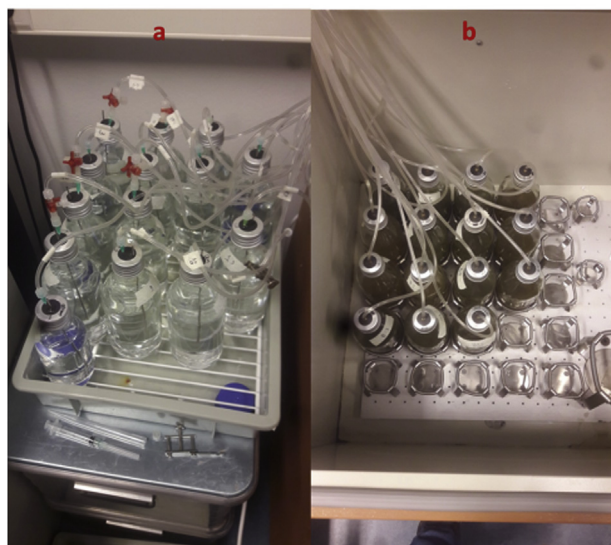


Fig. 2. experimental set-up (a) serum bottles, (b) flasks-reactors.

**Table 2**  
Experimental conditions of the batch tests.

Particular	Experimental design			
Exp. set	Co-digestion (based on VS)	Organic load (g VS <sub>added</sub> L <sup>-1</sup> )	Mass of substrate (g) added in the 300 mL bottle	Mass of co-substrate (g) added in the 300 mL bottle
CD	—	5	13.99	—
SM	—	5	7.02	—
CD:SM	1	5	6.99	3.51
CD:SSM	1	5	6.99	3.77
SM:SCD	1	5	3.51	6.16

The daily data of the biogas volume were normalized to normal millilitre (NmL) (dry gas,  $T = 0\text{ }^{\circ}\text{C}$ ,  $P = 760\text{ mm Hg} = 100\text{ kPa}$ ) according to the equation [11]:

$$V_N = (V \times 273 \times (760 - p_w)) / ((273 + T) \times 760)$$

where  $V_N$  is the volume of the dry biogas at standard temperature and pressure (NmL),  $V$  = recorded volume of the biogas (mL),  $p_w$  = water vapor pressure as a function of ambient temperature (mm Hg), and  $T$  = ambient temperature ( $^{\circ}\text{K}$ ).

All the experiments were carried out in triplicate and the results were expressed as means. The biogas measuring devices are simple water displacement bottles. The test ended when no more significant biogas production was observed, meaning no water noticed. The data analysis was conducted using Microsoft Excel. The daily data of the biogas volume were normalized to normal mL (dry gas,  $T = 0\text{ }^{\circ}\text{C}$ ,  $P = 101.3\text{ kPa}$ ). Biogas composition was determined at the end of the experimental period by using a micro-GC device and the methane potential was also expressed as NmL methane per gr of VS ( $\text{mL CH}_4\text{ g}^{-1}\text{ VS}$ ).

### 2.3. Analytical methods

TS and VS contents determined according to Standard Method 1684 [12]. Total alkalinity (TA) and total volatile fatty acids (TVFA) were measured according to the standard protocol EPA-430/9-77-006/March1977. COD was calculated using a test kit (Hach Lange GmbH) according to the manufacturer's instructions and a

spectrophotometer (DR/2010, Hach). A pH meter (HI991001, Hanna Instruments) was used to measure the pH. The biogas production was determined by means of water volume displacement. The concentrations of  $\text{CH}_4$  and  $\text{CO}_2$  were quantified by a micro gas chromatograph (single channel 2-stream selector system, Thermo Fisher Scientific) equipped with a chromatographic column (PLOT-U) with Helium as carrier gas at a total flow of  $10\text{ mL min}^{-1}$ . A gas standard consisting of 50% (v/v)  $\text{CH}_4$ , 20% (v/v)  $\text{CO}_2$  and 30% (v/v)  $\text{N}_2$  was used for calibrating gas chromatographic results. All the results represent the mean value of experiments conducted in triplicate with an accuracy of 10%.

### 2.4. Microbiological method DNA extraction and quantitative real time polymerase chain reaction (qPCR) analyses

Samples of 5 mL (from the first and last day) were frozen at  $-20\text{ }^{\circ}\text{C}$  until DNA extraction was conducted. DNA was isolated from the samples using the FastDNA SPIN Kit for soil (MP Bio-medicals) according to the protocol provided by the manufacturer [13]. DNA was stored  $-20\text{ }^{\circ}\text{C}$  after the isolation until qPCR method was performed using one mL of extracted DNA samples. DNA was eluted in 500  $\mu\text{L}$  milli-Q water. Eight primer sets (Table 3) were used for isolation of the 16S rRNA gene sequences of the following microorganisms: the order of Methanobacteriales, Methanococcales and Methanomicrobiales, the families Methanosarcinaceae and Methanosaetaceae, as well as the total bacteria and archaea according to the experimental procedures followed by Yu et al. [14].

qPCR was performed on DNA extracts of samples using iQ mastermix (Biorad) [15], Syto-9 as the fluorophore [16] at a final concentration of  $10\text{ }\mu\text{M}$  and primers at a final concentration of  $400\text{ nM}$  each. For the qPCR  $5\text{ }\mu\text{L}$  of template,  $5\text{ }\mu\text{L}$  primer mix, containing  $1.6\text{ }\mu\text{M}$  forward primer and  $1.6\text{ }\mu\text{M}$  reverse primer and  $10\text{ }\mu\text{L}$  iQ mastermix containing Syto-9 were mixed into a sterile 96-wells plate using an automated liquid handler (Beckman-Coulter Biomek 3000, Fullerton, CA, USA). All qPCR reactions were performed using an initial denaturation at  $94\text{ }^{\circ}\text{C}$  for 3 min, followed by 45 cycles of amplification with each cycle consisting of denaturation (30 s at  $94\text{ }^{\circ}\text{C}$ ), annealing (30 s at  $60\text{ }^{\circ}\text{C}$ ), elongation (30 s at  $72\text{ }^{\circ}\text{C}$ ) and a fluorescence measurement (5 s at  $80\text{ }^{\circ}\text{C}$ , excitation at 450–490 nm, emission at 515–530 nm). This was followed by a final extension at  $72\text{ }^{\circ}\text{C}$  for 5 min qPCR reactions were performed on an MJ Thermocycler PTC-200 with a Chromo 4 Detector (Biorad). For data analysis the software package MJ Opticon Monitor 3.1 was used with a fixed threshold setting of  $-1.6$ .

**Table 3**  
Primers used in this study.

Primer	Sequence 5' → 3' [Restriction Enzyme]	Length
ARC787F	ATTAGATACCCSBGTAGTCC	20
ARC1059R	GCCATGCACCCWCTCT	16
BAC338F	ACTCTACGGGAGGCAG	17
BAC805R	GACTACCAGGGTATCTAATCC	21
MCC495F	TAAGGCTGGGCAAGT	16
MCC832R	CACCTAGTYCGCARAGTTTA	20
MBT857F	CGWAGGGAAGCTGTTAAGT	19
MBT1196R	TACCGTCGTCACCTCTT	18
MMB282F	ATCGRTACGGGTGTGGG	18
MMB832R	CACCTAACGCRCATHGTTAC	21
MSL812F	GTAACGATRYTCGCTAGGT	20
MSL1159R	GGTCCCCACAGWGTACC	17
Msc380F	GAAACCGYATAAGGGGA	18
Msc828R	TAGCGARCATCGTTTACG	18
Mst702F	TAATCCTYGARGGACCACCA	20
Mst862R	CCTACGGCACCRACMAC	17



### 3. Results and discussion

#### 3.1. Characterization of bioreactors

To estimate the removal percentage of COD and VS, measurements were performed at the beginning and at the end of the essays. The characteristics of samples (start and end) in terms of VS, TS, COD, TA and TVFA are given in Table 4. The pH was stable which can be validated from the slight changes in TVFA. The COD and VS removal percentages are given in Table 5.

#### 3.2. Potential biogas production

The biogas potential of two kinds of animal slurries was determined without using inoculum. The product potential of the AD process was determined with respect to biogas and methane amounts (Table 6). Ideal anaerobic conditions as well as appropriate microbial and enzymes availability were taken into account during the experimental design. The addition of SM influences the digestion of CD and hence the biogas production yield.

Fig. 3 represents the cumulative biogas content of all digesters as function of time. CD had a better biogas yield reaching a total production of 52 NmL of biogas in 24 d which corresponds on 104 NmL biogas g<sup>-1</sup> VS added. The fact that the inoculum (anaerobic sludge) was not used is responsible for the long lag phase of biogas production. The lower biogas production of the mixture CD:SM could be due to antagonistic phenomena between the microorganisms of CD and SM.

Fig. 4 shows the daily biogas production of all reactors. The highest daily biogas production occurred in bioreactor with CD reaching a production peak of 10.2 NmL. In Table 6, biogas yields

and methane percentage are presented. The results clearly show the main contribution of the CD-based microorganisms on biogas production. The samples with SCD or without CD showed low biogas production potential. Data show that the most degradable is CD. A comparison between biogas yields and methane compositions for the 5 configurations is depicted in Table 6. Nevertheless, the quality of biogas (means percentage of methane) has to be considered. Among the five samples CD provided considerable better quality biogas reaching 64%.

#### 3.3. Microbial community analysis

qPCR analysis was performed to determine the total population of bacteria and archaea as well as to characterize the different methanogenic communities in the five different flasks at the beginning and end of the experiment. Total bacteria copy numbers were similar in the reactors except in the case of CD-SM and SM + SCD mixtures where it was a factor 10 lower. In general, total bacteria abundance was roughly a factor 10 higher at the beginning of the experiments. In the case of CD-SM and SM-SCD mixtures, total bacteria copy numbers were higher at the end (Fig. 5). A clear difference in methanogenic community composition and abundance was observed in digesters. Total archaea number shows an increasing trend during the experiment justifying the low biogas production (Fig. 3).

A majority of archaea was affiliated to Methanomicrobiales, and Mathanobacteriales and less to Methanosaetaceae and Methanosarcinaceae (Fig. 6). The Methanococcales abundance was below detection limit in all the samples. Also, it is noted that Methanosarcinaceae was found to increase in number in all the reactors when the experiment was ended. Methanomicrobiales, which was abundant in the beginning phase of SM-containing flasks, are reduced at the end indicating that methane production was limited. In the flasks with CD and CD-SSM the percentage of Methanobacteriales was increased indicating the higher biogas yields. The evaluation of the different methanogenic species revealed a clear view for co-digestion samples and most specifically for sheep addition. As for mono-digestion of SM, the total amount of Methanobacteriales in this treatment has a decreasing trend and reflects the lowest biogas, perhaps due to strong inhibition in mono-digestion of SM which hinders the growth of methanogens. The stable pH and TVFA values from the beginning and the end of the experiment (Table 4) indicate that higher amount of substrates can be treated. However, these findings cannot explain the direct correlation between the methanogenic communities and process conditions. The importance of co-substrate selection for biogas potential essays is important, as substrates contain specific microbial species which is related to different metabolic pathways during the AD as influences between microorganisms occur.

### 4. Conclusions

These essays highlighted the potential of cow dung and SM conversion to biogas through AD. Exploring the interactions between the different species of microorganisms in the samples and their effect into the conversion factor is necessary for further research. There are many different archaea endowed with an excellent capacity to produce methane, but for some unknown reasons, its exploration continues to be elusive. Problems connected with the use of SM are obvious from the low methane yield. We also need to explore alternative animal slurries which could be economically viable. From the experiment, it can be concluded that CD-based microorganisms make up the predominant factor for biogas production. The samples with CD, and CD-SM mixture resulted in 104 and 89 NmL biogas g<sup>-1</sup> VS respectively. Pre-

**Table 4**  
Physical and biochemical properties of samples at the beginning and end of the experiment.

	Samples*	pH	TS (g L <sup>-1</sup> )	VS (g L <sup>-1</sup> )	COD (g L <sup>-1</sup> )	TA (mg CaCO <sub>3</sub> L <sup>-1</sup> )	TVFA (mg acetate L <sup>-1</sup> )
Start	CD	7.25	7.1	1.6	145.0	240	524
	SM	7.16	7.8	1.8	299.1	231	530
	CD:SM	7.21	7.2	1.7	206.7	237	566
	CD:SSM	7.18	7.3	1.8	220.2	231	511
	SM:SCD	7.28	7.2	1.7	228.0	234	477
End	CD	7.02	6.4	1.2	94.5	239	488
	SM	7.19	6.8	1.6	244.8	231	478
	CD:SM	7.23	6.3	1.5	146.5	240	483
	CD:SSM	7.24	6.8	1.5	142.0	243	466
	SM:SCD	7.26	7.0	1.5	189.0	238	485

**Table 5**  
Removal percentages of VS and COD after 24 d.

Samples*	VS (%)	COD (%)
CD	22 ± 2	35 ± 4
SM	10 ± 1	18 ± 3
CD:SM	15 ± 3	29 ± 4
CD:SSM	19 ± 4	36 ± 5
SM:SCD	9 ± 1	17 ± 3

**Table 6**  
Biogas yield and percentage of methane in 24 d.

Samples	Biogas yield (NmL biogas g <sup>-1</sup> VS)	Methane in biogas (%)
CD	104	64
SM	8	54
CD:SM	77	61
CD:SSM	89	59
SM:SCD	23	56

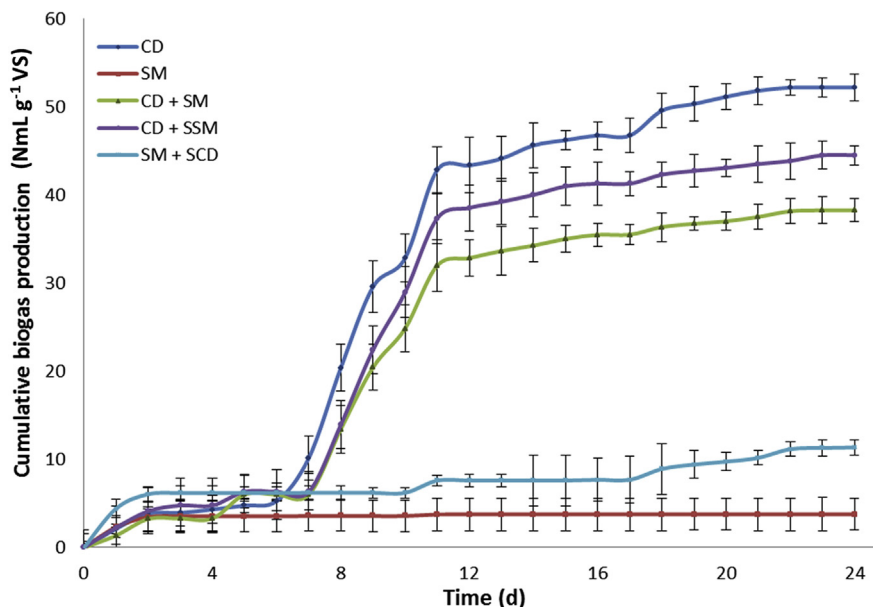


Fig. 3. BMP assay curves showing total biogas production (NmL) from 5 different samples. Error bars show standard deviations.

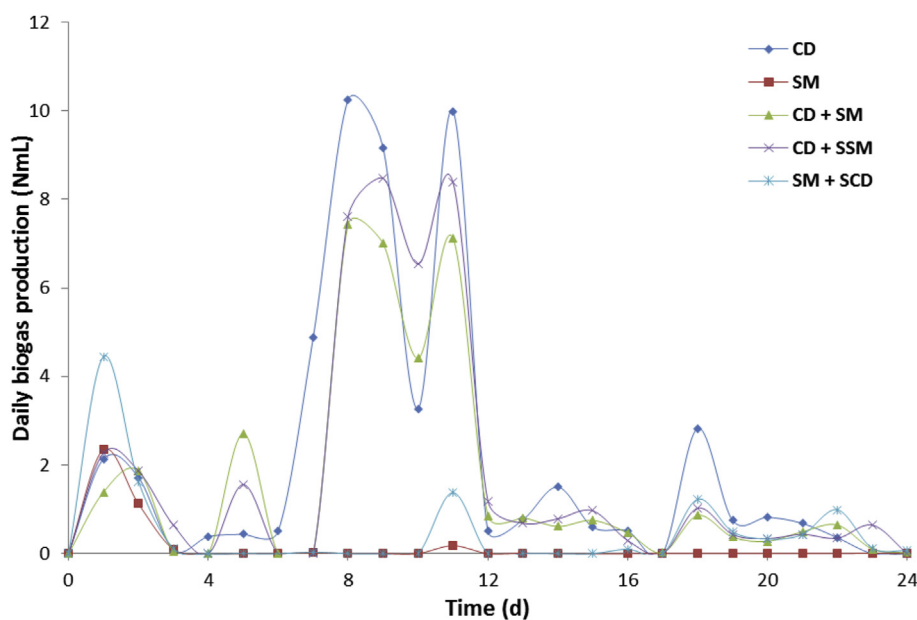


Fig. 4. BMP assay curves for a 24 d run showing daily biogas production (mL) from 5 different samples.

treatment can increase bio-methane production but economic evaluation is needed for these pre-treatments.

On the other hand, the samples containing SM-based microorganisms resulted in lower yields and the AD is probably inhibited from inactivity of the microorganisms. Compared with other previous experiments using CM and SM as substrate, our research offers a new perspective to understand the biochemical interactions within during the process, as the manure from these two typical ruminant animals contains many microbes themselves and it is important to figure out the microbial activities during the AD in order to have a better understanding of the whole process. Since AD is driven by several key microbes a good understanding of the

succession of these microbes may provide an answer to the correlating performance in practical application.

From the cumulative methane yield of different treatments, conclusion can also be drawn that thermal treatment used in this experiment can increase the methane yield of co-digestion CM and SM. Thus, thermal pretreatment may be an efficient way to treat manure waste in practical AD plants. Although there are several opportunities in the biogas sector, there are however challenges that cannot be ignored and barriers that have to be overcome. Considering the aforementioned facts, as well as the high availability of agro waste, the use of CD and SM as substrates for AD represents an option for large-scale applications.

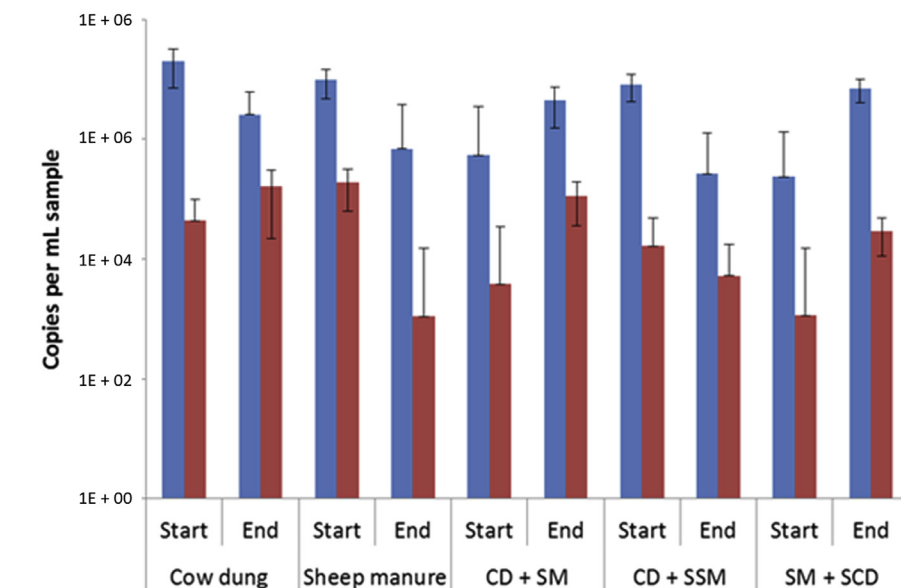


Fig. 5. Real-time qPCR results in the different samples, showing the total bacteria (blue) and total archaea (red). Error bars show standard deviations.

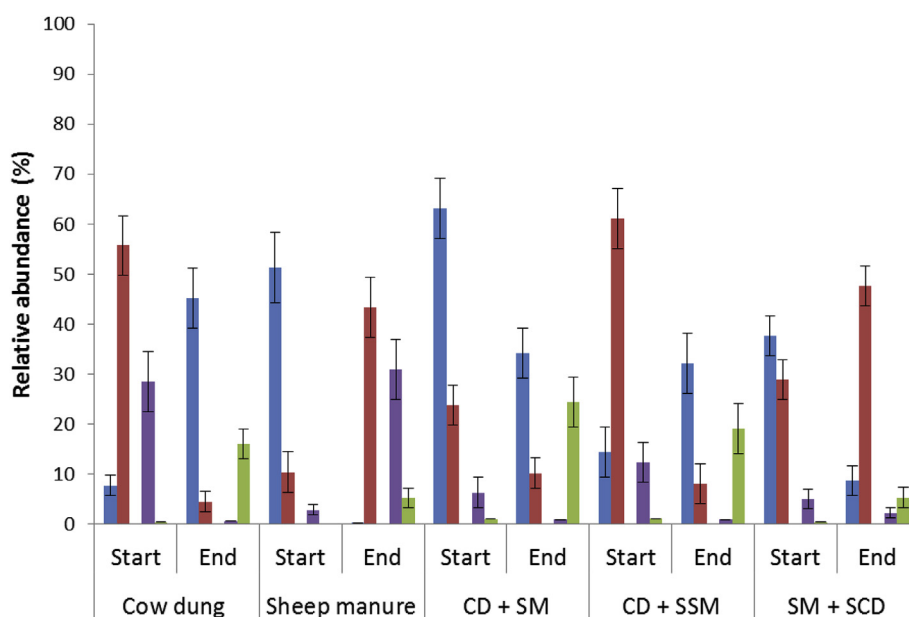


Fig. 6. Taxonomic classification of the methanogenic communities from qPCR results, showing Methanomicrobiales (blue), Methanobacteriales (red), Methanococcales (black), Methanosaetaceae (purple) and Methanosarcinaceae (green). Error bars show standard deviations.

## Acknowledgements

The authors would like to thank the providers of the substrates. The authors also kindly thank the Engineering and Technology Institute of Groningen at the University of Groningen for the use of the facilities.

## References

- [1] Coppolecchia D, Gardoni D, Baldini C, Borgonovo F, Guarino M. The influence on biogas production of three slurry-handling systems in dairy farms. *J Agr Eng* 2015;46:30–5.
- [2] Boscaro D, Pezzuolo A, Grigolato S, Cavalli R, Marinello F, Sartori L. Preliminary analysis on mowing and harvesting grass along riverbanks for the supply of anaerobic digestion plants in north-eastern Italy. *J Agr Eng* 2015;46:100–4.
- [3] Valenti F, Porto SMC, Chinnici G, Cascone G, Arcidiacono C. Assessment of citrus pulp availability for biogas production by using a GIS-based model: the case study of an area in southern Italy. *Chem Eng Trans* 2017;58:529–34.
- [4] dell'Antonia D, Cividino SRS, Carlino A, Gubiani R, Pergher G. Development perspectives for biogas production from agricultural waste in Friuli Venezia Giulia (Nord-East of Italy). *J Agr Eng* 2013;44:569–72.
- [5] Dinuccio E, Gioelli F, Cuk D, Rollè L, Balsari P. The use of co-digested solid fraction as feedstock for biogas plants. *J Agr Eng* 2013;44:153–9.
- [6] Menardo S, Balsari P. An Analysis of the energy potential of anaerobic digestion of agricultural by-products and organic waste. *Bioenerg Res* 2012;5: 759–67.
- [7] Perazzolo F, Mattachini G, Tambone F, Calcante A, Provolo G. Nutrient losses from cattle co-digestate slurry during storage. *J Agr Eng* 2016;47:94–9.
- [8] Cestonaro T, Costa MSSD, Costa LAD, Rozatti MAT, Pereira DC, Lorin HEF, et al. The anaerobic co-digestion of sheep bedding and ≥ 50% cattle manure increases biogas production and improves biofertilizer quality. *Waste Manag* 2015;46:612–8.

- [9] Alvarez R, Liden G. Low temperature anaerobic digestion of mixtures of llama, cow and sheep manure for improved methane production. *Biomass Bioenergy* 2009;33:527–33.
- [10] Pecorini I, Baldi F, Carnevale EA, Corti A. Biochemical methane potential tests of different autoclaved and microwaved lignocellulosic organic fractions of municipal solid waste. *Waste Manag* 2016;56:143–50.
- [11] Dinuccio E, Balsari P, Gioelli F, Menardo S. Evaluation of the biogas productivity potential of some Italian agro-industrial biomasses. *Bioresour Technol* 2010;101:3780–3.
- [12] USEPA. Method 1684: Total, Fixed, and Volatile Solids in Water, Solids, and Biosolids. Washington, DC: US Environmental Protection Agency; 2001.
- [13] MPB. FastDNA® SPIN Kit for Soil. Solon, OH: MP Biomedicals.
- [14] Yu Y, Lee C, Kim J, Hwang S. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnol Bioeng* 2005;89:670–9.
- [15] Bio-Rad. Hercules, CA. <https://www.bio-rad.com/>.
- [16] Monis PT, Giglio S, Saint CP. Comparison of SYTO9 and SYBR Green I for real-time polymerase chain reaction and investigation of the effect of dye concentration on amplification and DNA melting curve analysis. *Anal Biochem* 2005;340:24–34.